Complexation Reactions Between Trace Metals And Specific Functional Groups In Natural Organic Matter From Estuarine Waters

Peter H. Santschi
Department of Oceanography, Texas A&M University
Galveston, TX 77553-1675
phone: 409-740-4476; fax: 409-740-4786; email: santschi@tamug.tamu.edu
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LONG-TERM GOALS

To gain a better understanding of the role of colloidal organic macromolecules and inorganic colloidal microparticles in the cycling, speciation and bioavailability of trace elements in coastal waters.

SCIENTIFIC OBJECTIVES

1) Concentration determination and isolation of purified fractions of natural thiols and natural acid polysaccharides for trace metal complexation and structural experiments.
2) Determination of conditional stability constants for the complexation of selected B metal ions (such as Ag, Hg, Cd, Cu, Pb) to specific thiolic functional groups in estuarine organic matter, and of A (e.g., Cs, Ba, Th) and transition metal (e.g., Fe, Cr, Zn, Co) ions to fibrillar acid polysaccharides (PS).
3) Determination of the physical form and chemical structure of these biopolymers.
4) Determination of stability of these complexes towards light, oxygen and microbial degradation.

APPROACH

In addition to the PI, Assistant Research Scientist Dr. Liang-Saw Wen worked on this project for four months, and left in Feb. 1, 1999, to take on the position of the Director of a Water Chemistry Laboratory in Singapore. Dr. Chin-Chang Hung, post-doctoral research associate, started to work on this project on August 1, 1999. In addition to these scientists, graduate student Degui Tang, who is scheduled to defend his Ph.D. dissertation in spring 2000, graduate student Kent Warnken (in part), and research assistant Susan Gonzalez (left at beginning of 1999) have been supported on this project during the last year.

Approaches used in this project include: 1) Determination of high and low molecular weight (LMW and HMW) thiol and polysaccharides (PS) concentrations in estuarine waters of Galveston Bay, followed by collection and physical and chemical separation of colloids are into PS, protein, and humic fractions. 2) Use of AFM to check endmember biopolymer size and abundance according to Santschi et al. (1998). 3) Use of HPLC to separate, identify and measure the concentration of different thiols and PS in NOM of different sizes, using fluorescence and UV/Vis or PAD (Pulsed Amperometric) detection. 4) Experimental determination of conditional stability constants of selected thiolic functional group containing molecules in proteins for B- and borderline metals using stable and radioactive metal ion additions to separate colloid fractions, followed by HPLC separation and detection of sulfhydryl groups by fluorescence, UV/Vis and PAD (Pulsed Amperometric Detector), and trace metals by either GF-AAS, ICP-MS or Gamma detection. 5) For isolated acid PS, a similar approach as in point 4) will

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determine conditional stability constants for A- and borderline metals. 6) The strategy for 4) and 5) will be slightly different in that we will use PS enriched samples for complexation and other experiments, while for proteins, we will use both pre- and post-column additions of trace metals into the bulk colloids samples followed by separation of individual fractions by HPLC. 7) Test the stability towards biodegradation or coagulation of colloids and separated PS fraction from retentates or rehydrated freeze-dried material. 8) Determine long-term stability of metal-bound thiols towards air oxidation or light.

WORK COMPLETED

None of the major objectives have been completed. However, we have made considerable progress in the following areas: 1) Publication of manuscripts submitted during the previous grant period. 2) Finished new calibration experiments to test the cross-flow ultrafiltration technique during this grant period, and submitted a manuscript for publication on this work, which has been accepted. 3) Made considerable progress in thiol analysis and isolation using HPLC during this grant period, and submitted a manuscript for publication. 4) Made considerable progress in PS isolation and analysis.

RESULTS

A) Further cross-flow ultrafiltration (CFUF) calibrations

One of the main goals of this project is to isolate colloidal macromolecular weight dissolved organic matter (DOC) for laboratory experiments to study the complexation between metals and colloidal organic matter in estuarine waters. Therefore, we have carried out controlled laboratory experiments to further calibrate our ultrafiltration systems and to investigate the retention and permeation behavior of macromolecules during ultrafiltration using molecular probes and radioactive metals spiked to natural seawater. Laboratory results from molecular probes show that significant fractions (>40%) of low molecular weight (LMW) molecules (0.5 kDa rhodamine 6G and 0.6 kDa glutathione) are retained by a 1 kDa ultrafilter membrane even under high concentration factors, CFs (e.g., >50). The percentage of high molecular weight (HMW) molecules passing through the 1 kDa membrane decreases rapidly with increasing size or MW. However, permeation of a 10 kDa dextran through the 1 kDa membrane becomes negligible (<0.6%). Thus, the permeation of HMW molecules is minimal even under high CFs while the retention of LMW molecules under lower CFs can give rise to an overestimate of the colloidal fraction. Natural DOC and LMW metals follow the same ultrafiltration behavior and consistently show an increasing concentration in the permeate with increasing CFs. Standard dextrans, rhodamine 6G and natural DOC have a recovery of 92-95% while losses of glutathione and vitamin B_{12} to the membrane can be significant. Sorptive losses and the overall mass balance of molecules are thus dominated by their physicochemical properties but not molecular weights. Most retained LMW DOC can be further removed during diafiltration whereas loss of HMW DOC during diafiltration is minimal. Since the retention of LMW molecules dominates the permeation of HMW molecules, a high concentration factor (>40) is found to be optimal for isolating marine colloids by ultrafiltration.

B) Trace metal binding to colloids

Desalted and freeze dried isolated marine colloidal material (1 kDa - 0.2 μm) from estuarine and marine waters was further analyzed for its trace metal (Cu, Pb, Zn, Cd, Co, Ni, Cr, Be, Fe, Al, Mn, V, Ba, and Ti) composition using acid digestion followed by ICP-MS analysis. It was found that these desalted and freeze dried colloids contain different levels of trace metals. Concentrations of these metals in marine colloids ranged from <0.1 to ~50 μg/g colloidal matter, except for Fe which generally had a concentration > 120 μg/g. Most metals (Cu, Pb, Zn, Ni, Al, Mn, V, and Ti) had an average concentration >1 μg/g while concentrations of Cd, Co and Be were usually <1 μg/g. Metal concentrations (μg/g) in isolated colloids were, in general, higher in Galveston Bay than in the Gulf of Mexico, suggesting either high abundance of trace metals in estuarine waters or high binding efficiency of estuarine organic materials. Most importantly, colloids had a metal composition and metal/organic carbon (Me/C) ratio similar to those of humic substances and marine plankton, suggesting that marine colloids largely originate from planktonic sources and are composed of predominately organic components. The relative affinity of metals for marine colloids followed the sequence of Cu > Cd > Ni > Zn > Co > Fe, Mn, which is similar to the Irvings-Williams order except for Cd.

C) Separation and detection on Metal-thiol complexes by HPLC: problems and implications

Natural samples and standard thiol compounds were used to investigate HPLC column separation and analysis of thiol-metal complexes. Metal concentrations were measured by GFAAS and thiol concentrations were determined by SBDF fluorescence detection. The approach using pre-column derivatization of thiols and measuring concentrations of metals co-eluted with thiol peaks proved to be unsuccessful. The derivatization of thiol-metal complexes by SBDF leads to displacement of some metals (e.g., Ag) by SBDF from the thiol-metal complexes, because the SBDF is covalently bound to –SH group in thiols (Tang et al., 1999). The metal eluted from the column is therefore unrelated to the thiol-SBD derivatives. As an example, Ag concentrations increase during the elution following the acetonitrile increase in a gradient run of HPLC. The post-column determination approach is more promising. During reversed phase separation, Ag complexed by low-molecular-weight thiols (glutathione or cysteine) could not be eluted from a C18 column. Similar results were observed for Ag-phytochelatin complexes (Mehra, et al. 1996). On the other hand, Ag complexed by a high-molecular-weight protein (metallothionein) could be eluted from the C18 column. However, in the current experimental protocol, silver-bound thiols could not yet be detected when using the current SBDF method. Other studies showed that Hg (II) and Cu(I) treated metallothioneins are difficult to detect by the SBDF method (Miyairi et. al., 1998). Size exclusion chromatography was also carried out on a TSK PW2500xl column, which is a polymer based column with an exclusion limit of about 6kDa for globular proteins. Again, only Ag complexed by metallothionein was eluted out. When natural colloids (< 1kDa) were applied to the column, the glutathione eluted at the same retention time (at 15 minute) as natural Fe, Cu and Cd, while Ag concentration was below the current detection limit. If these same metals are present in ionic forms, they are not eluted until after 60 minutes under the same experimental conditions, using a TRIS buffer. This suggests that thiols bind certain colloidal trace metals from Galveston Bay.

D) Thiol distributions in Galveston Bay

The distribution of aquatic thiols was studied by both voltammetry and HPLC with SBDF fluorescence detection. The total dissolved (≤0.45µm) thiol concentrations determined by square wave voltammetry ranged from 10 to 120 nM (as glutathione) in Galveston Bay. This result determined by the voltammetry method also includes metal sulfide species which need to be determined separately. The HPLC results showed that glutathione, cysteine, n-acetyl-cysteine, phytochelatins and others are all present in the estuarine water. The glutathione and an unknown thiol showed peaks emerging from the baseline, and the glutathione concentration ranged from 0.5 to 6 nM. The concentrations of cysteine were higher, ranging from below detection limit (~5 nM) to 22 nM. In lower Galveston Bay (Houston Ship Channel), thiols showed a conservative distribution along the salinity gradient; while in the upper Galveston Bay, dissolved (≤0.45µm) thiols were more or less uniformly present in surface waters. The total thiol concentration determined by HPLC detection is about half of that by the voltammetric measurement, which is in reasonable agreement, as the HPLC was optimized only for low-molecular-weight thiol detection. Dissolved (metal) sulfides were also determined by the methylene blue Cline method after a solid phase extraction on the Waters tC18 cartridge, with concentrations ranging from 2-4 nM. This result shows that in estuarine waters, contrary to freshwaters, thiol concentrations are orders of magnitude higher than those of Cline-reactive sulfides. This makes it likely that dissolved thiols and sulfides control the metal speciation in estuarine waters.

E) Polysaccharide separation and distributions in Galveston Bay

Samples were collected using a 1kDa cross-flow ultrafiltration apparatus to process 500 liters of Gulf of Mexico coastal surface water which had been prefiltered in the field through a 0.4µm filter. COM was then dialyzed to remove sea salt and the final concentrate was freeze dried. A fraction of the freeze dried solid was then redissolved in dH_2O and mixed with anhydrous ethanol at a 4 to 1 ratio. The resultant precipitate was collected by centrifugation and the overlying ethanol decanted off. The precipitate was redissolved in dH_2O and reprecipitated in ethanol twice more before the final precipitate was collected and freeze dried. The fraction of organic carbon content which consists of PS was over 45% in the precipitated material. The total dissolved and colloidal PS in surface waters in Galveston Bay were determined based upon oxidizing the free reduced sugars with ferricyanide reaction (Myklestad et al. 1997). The dissolved PS in Galveston Bay ranged from 24 to 99 µmol glucose-C L^{-1} and ranged from 7 to 21%, average 15%, of DOC. The colloidal PS in Galveston Bay varied from 13 to 60 µmol glucose-C L^{-1}, and accounted for 13 to 36 %, average 24%, of COC. The highest concentrations of total and colloidal PS were found in the low salinity regimes of Galveston Bay and total and colloidal PS decreased with the increased salinity. The fraction of high molecular weight PS in COC is significantly higher than that of total dissolved PS in DOC. The results suggest PS are an important component of the COC in Galveston Bay.

F) Polysaccharide binding to metals

Using stirred-cell ultrafiltration, conditional stability constants were determined for the complexation of different metals onto natural organic matter, NOM, and PS rich natural organic mater (PRNOM) as a partitioning coefficient, defined here as K_d (l/kg). K_d is the ratio of particle- (or colloid-) bound metal to dissolved metal normalized to the particle (or colloid) concentration at ambient conditions. The separation between sorbed and dissolved was achieved using a 1 kDa molecular weight

cut off stirred cell ultrafiltration. For the metals tested there was an increase in the partitioning coefficient to PRNOM by a factor of 2-10 over the partitioning of the metal with bulk COM. Specifically, Fe, Zn, Pb and Am have a log K_d value close to 6, with a range from 5.97 - 6.07 for the bulk COM. Log K_d increases to nearly 7 for the PS-rich NOM for these metals, with a range from 6.40 - 7.16. For Mn, Cs, Cd, Ba, Co and Pu the log K_d for bulk COM is lower than for the other metals, with a value close to 4 and a range from 3.76 - 5.52.

G) Alternative CFUF methods

Cross calibration of the above mentioned ultrafiltration techniques with small volume techniques such ultracentrifugation using Microcon concentrators with molecular weight cut-offs (MWCO) of 3, 30 and 100 kDa have begun. Initial work suggests these filters can be used quantitatively with good recovery of the filtered samples (97-103%). Results from the 3kD ultrafilter show that 7000xg results in a concentration factor of 6-7 while speeds up to 14,000xg gave concentration factors of about 60 and lower recoveries (80%). Once developed, this small volume method will be used to determine trace metal (Ag, Cd and Pb) phase fractionation in porewaters and the role colloids play in the release of these metals from sediments.

IMPACT/APPLICATIONS

1) We were able to make the following recommendation to the research community: high concentration factors and/or diafiltration are needed to minimize the entrainement of low molecular weight molecules into the colloidal fraction when using cross-flow ultrafiltration. 2) The fact that many publications resulting from this research are frequently cited in the literature suggests that the results from this research are utilized by other researchers in the field.

TRANSITIONS

Our analytical methodology, hardware and software have been used in a NSF-funded study of the interactions between Th isotopes and acid PS to improve the POC/^{234}Th ratio method of new production.

RELATED PROJECTS

This project benefited from other programs which investigate marine colloids and which are directed by Peter H. Santschi. They include: 1) NSF - OCE, “Relationship of Th(IV) speciation to scavenging in marine environments”; 2) Kaiser-Hill/Dept. of Energy, “Actinide Migration Studies at the Rocky Flats Environmental Technology Site”; 3) Texas Advanced Research Program, "Reconstruction of Terrestrial ^{129}I Inputs into Marine Environments"; 4) Texas Seagrant, "Bioavailability of colloid-associated metals to estuarine bivalves"; 5) National Science Foundation, "A Collaborative Proposal on the Interaction of Th(IV) with Organic Compound Classes of Marine Organic Matter". This ONR funded project benefited also from collaborations with Drs. J. Buffle, K. Wilkinson, and E. Balnois, Dept. of Analytical Chemistry, University of Geneva, Switzerland, for the AFM and TEM work on COM, and with Dr. John Cantois, Specells, Inc., Houston, TX, for the HPLC work with Ag-thiols.

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